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Comparison of methods for isolating *Salmonella* bacteria from faeces of naturally infected pigs

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P.R. DAVIES, P.K. TURKSON, J.A. FUNK, M.A. NICHOLS, S.R. LADELY AND P.J. FEDORKA-CRAY. 2000. A series of experiments was conducted using faecal samples collected from commercial swine farms to evaluate the effects of variation in methods used for the detection of *Salmonella* bacteria. The primary objective of the studies was to compare the protocols routinely used in two laboratories in the USA. The studies included five experiments comparing the enrichment protocols used routinely in the respective laboratories (Method 1: 10 g faeces—buffered peptone water (BPW) pre-enrichment—selective enrichment in Rappaport/Vassiliadis (RV) broth; Method 2: ~1g faeces—primary enrichments in tetrathionate and Hajna GN broths—secondary enrichment in RV broth). The effects of enrichment temperatures (37 *vs* 42 °C) using RV broth (two experiments) and delayed secondary enrichment (four experiments) were also evaluated. Direct comparison of Method 1 and Method 2 indicated comparable results. However, when compared using faecal samples of equal weight, the Method 2 enrichment protocol was more sensitive for detecting *Salmonella* bacteria than the Method 1 protocol. Enrichment in RV at 42 °C was superior to 37 °C, particularly for samples that were pre-enriched in BPW. Delayed secondary enrichment increased detection of *Salmonella* bacteria in swine faeces. These results highlight the imperfect sensitivity of culture methods, and the need for researchers to consider the sensitivity of bacteriological methods in the design and interpretation of the results of epidemiologic studies based on faecal culture

INTRODUCTION

Public concerns about food safety have drawn attention to the desirability of reducing the prevalence of food-borne pathogens in food animal populations. Salmonellosis was ranked by the United States Department of Agriculture to be the most important food-borne disease in the USA linked to red meat and poultry products (Anon. 1995). A general conclusion of that review was that there is inadequate knowledge relating to the epidemiology of food-borne agents in animal production, and the need for a better understanding of the epidemiology of infections caused by *Salmonella* has been specifically emphasized (Tauxe 1991). The implementation of a national *Salmonella* control programme by the swine industry in Denmark, the leading pork exporting nation, has also increased the importance of *Salmonella* control to the US swine industry, which has recently become a net exporter of pork products (Davies 1997; Mousing *et al*. 1997).

Despite considerable advances in newer diagnostic methods, conventional bacteriology remains the foundation of epidemiological studies of *Salmonella* bacteria (Lax *et al*. 1995). It has been suggested that there may be more media and culture methods for the isolation of *Salmonella* bacteria than for any other bacteria (Waltman 1998). A plethora of studies have compared microbiological techniques for isolating *Salmonella* bacteria from diverse materials, and conflicting findings among studies abound (Harvey and Price 1981; D'aoust 1989; Busse 1995). The scope of this methodological dilemma was highlighted in a survey of US veterinary laboratories culturing poultry tissue and environmental samples for *Salmonella* bacteria. No two of the 74 respondent laboratories used identical protocols, and the authors reported the use of 17 different selective

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enrichment media or combinations of enrichment media, considerable variation in the duration and temperature of selective enrichment, and the use of 14 different plating media (Waltman and Mallinson 1995). The implications for interpreting data from different laboratories are obvious, and the authors argued there was a critical need for some standardization of laboratory protocols. Similarly, the need to standardize procedures, or quantify differences among procedures, so that results from different studies can be compared was a common conclusion from workshop sessions at the First International Meeting on the Ecology of *Salmonella* in Pork Production in 1996 (Anon. 1996).

Further impetus to conduct the methodological studies reported here came from disparate estimates of the prevalence of *Salmonella* bacteria in finishing pigs reported in studies by the authors' respective groups. In 1995, a survey of faecal shedding of *Salmonella* bacteria by finishing hogs on 152 farms in 16 states found 6·0% of 6655 individual samples and 38% of farms had *Salmonella* bacteria (Anon. 1997). In contrast, in a study of 29 finishing farms in North Carolina in 1994/95, 25% of 2288 faecal samples were found to contain *Salmonella* bacteria, including at least one culture-positive faecal sample on 24 (83%) farms (Davies *et al*. 1997). Among other differences in the design of these studies, the use of different laboratory protocols may have contributed to the varied findings. The primary objective of the studies reported here was to compare the protocols routinely used in the two laboratories which have collectively participated in most studies of *Salmonella* in swine in the USA published recently. Additional objectives were to compare temperatures for enrichment in Rappaport–Vassiliadis broth (37 *vs* 42 °C) and to evaluate further delayed secondary enrichment (DSE) with swine faecal samples (Nietfield *et al*. 1998; O'Carroll *et al*. 1999). It is hoped that these findings will provide some basis for more meaningful interpretation of earlier and other ongoing epidemiological studies in the USA, and a framework for developing standardized or comparable laboratory protocols for researchers.

MATERIALS AND METHODS

All experiments were conducted using swine faeces collected from individual pigs on commercial swine farms in North Carolina, USA. All farms had been shown to have *Salmonella* bacteria in earlier studies. The farms used for a given experiment were selected by convenience, when samples were to be collected as part of concurrent epidemiological studies. Within experiments, all comparisons of isolation methods were made on paired samples collected *per rectum* from individual pigs, or collected from pigs observed defaecating.

The standard protocols used in the respective laboratories have been described elsewhere (Davies *et al*. 1998; Fedorka-Cray *et al*. 1998) and are outlined schematically in Fig. 1. Laboratories used conventional biochemical screening of isolates prior to serotyping by the National Veterinary Services Laboratories (NVSL), Ames, IA, USA. In this laboratory, isolates were screened biochemically with triple sugar iron (TSI) and Christensen's urea agar slants. In the second laboratory, isolates were screened with TSI and lysine-iron agar (LIA), and were also serogrouped before being sent to NVSL.

A series of 11 experiments was performed: four experiments compared the pre-enrichment/enrichment protocols of the respective methods, one experiment compared the two isolation methods in both laboratories, two experiments compared enrichment of RV broth at 37 and 42 °C, and four experiments evaluated delayed secondary enrichment in RV broth.

Comparison of enrichment protocols

Four experiments were performed to compare the two pre-enrichment/enrichment protocols. Because of the difference in faecal sample weight (1 g *vs* 10 g) in the two standard protocols, and having previously demonstrated a marked effect of sample weight on detection of *Salmonella* bacteria using Method 1 (Funk *et al*. 2000), an additional treatment was included to enable comparison of the protocols using samples of equal weight. Faecal samples were collected from 459 pigs on four commercial farms (one farm per experiment) in North Carolina (96–121 pigs per farm). Samples were placed into sterile plastic bags and processed the same day. Small animal faecal loops were used instead of swabs to place small volume samples in tubes. One loopful of faeces from each sample was placed into each of three culture tubes containing 9 ml GN Hajna broth, sodium tetrathionate broth (TTB) or 2% buffered peptone water, respectively. The fourth method involved diluting approximately 10 g faeces in 2% BPW solution in a 1:9 ratio by weight in plastic bags (Method 1). All samples (excluding those in TTB) were incubated overnight at 37 °C. For the TTB samples, the incubation period was 48 h. A 0·1 ml aliquot was transferred to 9·9 ml RV broth and incubated at 37 °C (for the Hajna and TTB samples), or at 42 °C (for the loopful and 10 g BPW samples), for 24 h. Each RV broth culture was streaked on xylosine–lysine–tergitol-4 agar and brilliant green sulphur agar plates, which were incubated overnight at 37 °C. One colony per plate with morphology consistent with *Salmonella* was screened biochemically, and isolates presumptively identified as *Salmonella* bacteria were submitted to NVSL for serotyping.

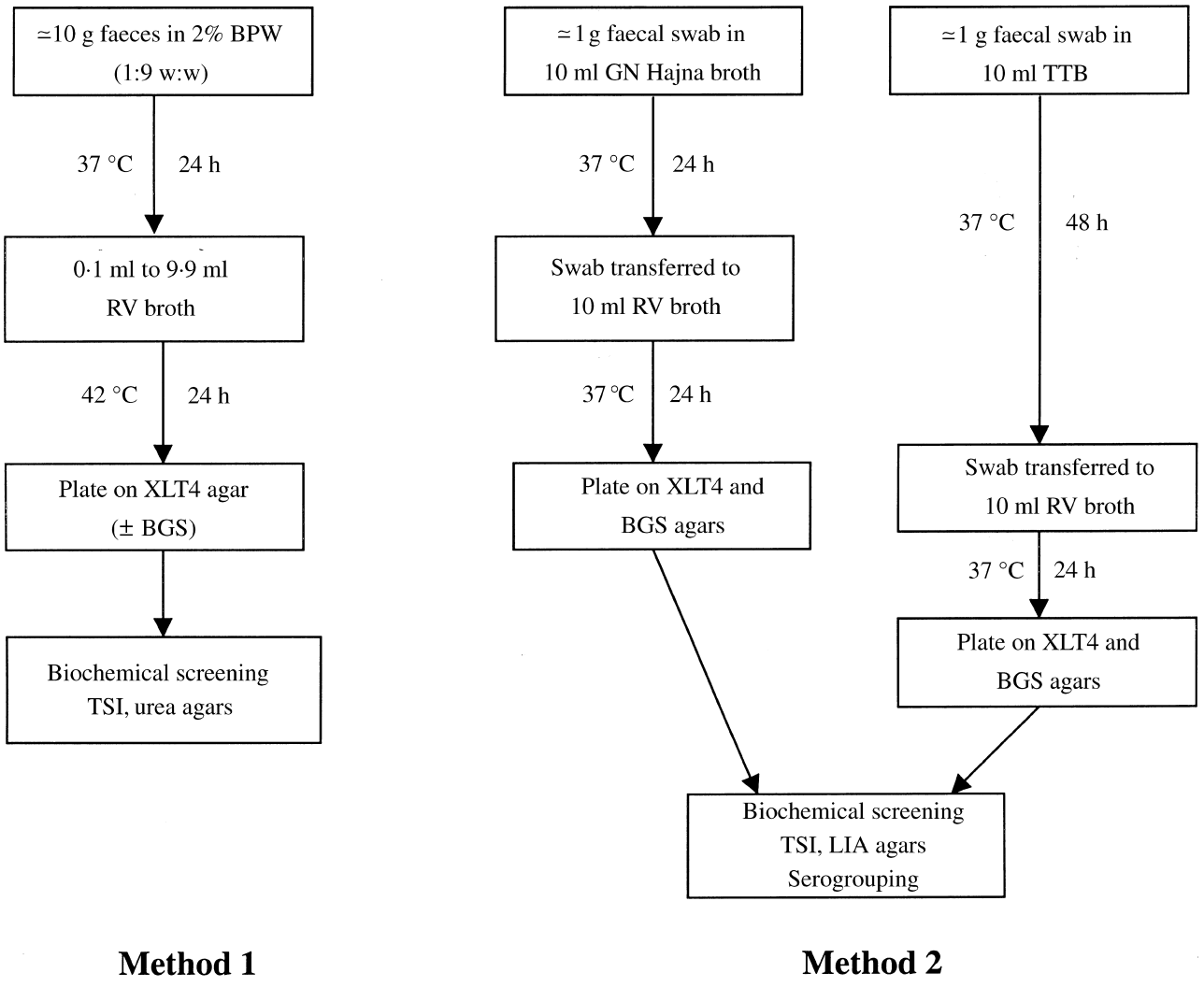

Fig. 1 Schematic outline of routine methods used in the respective laboratories to isolate *Salmonella* from swine faeces

Comparison of Method 1 and Method 2 in both laboratories

Paired faecal samples were collected from 96 pigs at a finishing farm in North Carolina. The samples were transported to this laboratory at ambient temperature, where paired sets were either stored overnight at 4 °C (NC samples) or transported on ice to the participating laboratory in Athens, GA, USA. The samples were processed with both isolation methods at each laboratory. Transfer methods and volumes into RV broth were as described above for the respective methods. Broths from Method 1 were streaked on XLT4 plates only, while broths from Method 2 were streaked on both XLT4 and BGS plates (in accordance with current standard procedures). No attempt was made to standardize procedures for colony selection and confirmation.

Comparison of 37 and 42 °C enrichment in RV broth

Two experiments were conducted to compare enrichment in RV broth at 37 and 42 °C. The first experiment (128 sows, 10 boars at one farm) followed the Method 1 protocol but included an additional treatment using 37 °C incubation (air incubator) as well as 42 °C incubation in a waterbath. In the second experiment (96 finishing pigs), both pre-enrichment of 10 g faeces in BPW (as in Method 1) and enrichment of a loopful of faeces in TTB for 48 h

(Method 2, excluding Hajna broth) preceded RV (37 and 42 °C) enrichment. For both BPW and TTB, 0·1 ml broth was transferred to 9·9 ml RV broth by pipette. Hajna broth was not included because of logistic constraints and the fact that TTB enrichment usually contributes the majority of isolates obtained with Method 2. Plating was performed on XLT4 agar.

Delayed secondary enrichment

Delayed secondary enrichment (DSE) was evaluated in four experiments, one of which has been reported separately (O'Carroll *et al*. 1999). In all experiments, RV tubes from the primary enrichments were stored at room temperature (20–22 °C) for 4 days, after which a 0·1 ml aliquot was pipetted to 9·9 ml RV broth and incubated in a 42 °C water-bath for 24 h (DSE). These samples were streaked on XLT4 agar plates and processed in an identical manner to the primary enrichment samples. As the 4 day delay used in the initial study was arbitrary, in two experiments, delays of 0 (secondary enrichment without delay), 4 and 8 days before secondary enrichment was performed were also compared .

Statistical analysis

Comparisons of the proportion of positive samples were tested using McNemar's chi-square test for paired samples. Agreement between results of methods and laboratories was evaluated using the kappa statistic (Fleiss 1981).

RESULTS

Comparison of enrichment protocols

Among the four experiments, *Salmonella* bacteria were cultured from 111 (24·2%) of the 459 faecal samples (Table 1). *Salmonella* bacteria were not isolated in experiment 3, and the prevalence of samples containing *Salmonella* bacteria ranged from 0% (experiment 3) to 65% (experiment 4). Overall, the proportion of all positive pigs detected with Method 1 (94 of 111; 84·7%) was very similar to Method 2 (91; 82·0%). For individual samples across the three experiments in which *Salmonella* bacteria were detected, the two protocols showed excellent agreement (89% agreement, kappa = 0·72). However, percentage of agreement (80–98%) and kappa (0·59–0·88) varied considerably between individual experiments. When compared on samples of equal weight (faecal loops), both enrichment in TTB (74%) and in Hajna (48%) alone yielded a higher proportion ($P < 0·001$) of positive cultures than did pre-enrichment in BPW (23%). Faecal sample weight had a marked effect on results with the Method 1 protocol. Enrichment in TTB alone yielded 90·2% of positive results with Method 2, compared with 57% from enrichment in GN Hajna.

Table 1 Number of culture-positive samples for four culture protocols in four experiments (n = number of faecal samples evaluated)

Treatment	Experiment 1 $n = 121$	Experiment 2 $n = 121$	Experiment 3 $n = 96$	Experiment 4 $n = 121$	Total $n = 459$
Method 1: BPW-RV					
Loop of faeces	9	6	0	11	26
10 g faeces*	14	8	0	72	94
Method 2: TTB/Hajna – TTB-RV					
Loop of faeces –TTB	13	10	0	59	82
Loop of faeces –Hajna	8	6	0	39	53
Loop–Hajna or TTB†	15	10	0	66	91
Positive (%) on any culture	20 (16·5)	10 (8·3)	0 (0)	81 (66·9)	111 (24·2)
Serotypes isolated	*typhimurium*	*typhimurium*‡ *derby* *new brunswick*		*typhimurium* *typhimurium*‡ *brandenburg* *heidelberg* 4,12:i monophasic	

*Standard method in Davies Laboratory.
†Standard method in Cray Laboratory (samples positive with either TTB or Hajna enrichment).
‡var. *copenhagen*.

Table 2 Number of cultures (pigs) found to contain *Salmonella* bacteria using Method 1 and Method 2, and serotypes identified at the two laboratories

	Method 1	Method 2	Method 1 or 2	Serotypes*
Lab. 1	24	29	37	*bietri*, *derby*, *give*, *heidelberg*, *kentucky*, *mbandaka*, *typhimurium*, *typhimurium* var. *copenhagen*
Lab. 2	33	26	37	*bietri*, *derby*, *give*, *heidelberg*, *typhimurium*, *typhimurium* var. *copenhagen*, untypable
Lab. 1 or 2	38	35	46	

*Two serotypes were isolated from individual pigs in laboratory 1 and from four pigs in laboratory 2.

Comparison of Methods 1 and 2 in both laboratories

Salmonella bacteria were cultured from 46 of the pigs sampled (Table 2), of which the individual laboratories each identified *Salmonella* bacteria in 37 pigs (81% agreement, kappa = 0·60). A total of nine serotypes were identified, with *Salm. bietri* the predominant serotype among isolates from both laboratories. *Salmonella* bacteria were isolated from slightly more samples using Method 1 (37) than Method 2 (35), but the difference was not statistically significant ($P = 0{\cdot}49$). Using the kappa statistic as a measure of agreement, the results indicated similar agreement between laboratories within methods (kappa = 0·55, 0·59) and between methods within laboratories (kappa = 0·45, 0·61). Combining results from both laboratories, enrichment in TTB alone yielded 82% (45 of 55) of positive results with Method 2, compared with 47% (26 of 55) from enrichment in GN Hajna. Pooling the data from 70 samples in this experiment and 255 samples in the four experiments described above, from which *Salmonella* bacteria were isolated on either XLT4 or BGS agars, typical *Salmonella* colonies were not present on XLT4 plates of 28 (8·6%) samples, and typical colonies were not present on BGS plates of 37 (11·4%) samples.

Comparison of 37 and 42 °C enrichment in RV broth

In experiment 1, seven serotypes of *Salmonella* bacteria were isolated from 19 of 136 faecal samples. *Salmonella* bacteria were isolated from more samples ($P = 0{\cdot}02$) incubated in RV broth at 42 °C (16) than samples incubated at 37 °C (seven). All seven serotypes were isolated from samples incubated at 42 °C, compared with four of the seven serotypes isolated following 37 °C incubation. For the four samples from which *Salmonella* bacteria were isolated at both temperatures, the same serotypes were isolated in all instances.

In experiment 2, RV enrichment at 37 and 42 °C following either BPW pre-enrichment or TTB enrichment resulted in detection of *Salmonella* bacteria in 50 of 96 (52%) faecal samples. Serotypes isolated were *Salm. muenchen* (87), *Salm. typhimurium* (39) and *Salm. typhimurium* var. *copenhagen* (15). *Salmonella* bacteria were isolated from more samples (76 of 192; 39·6%) incubated at 42 °C than at 37 °C (65 of 192; 33·9%). This difference was primarily attributable to the samples pre-enriched in BPW (44 positive at 42 °C; 37 positive at 37 °C; $P < 0{\cdot}05$) rather than samples enriched in TTB (32 *vs* 28 samples positive; $P = 0{\cdot}10$). Many of the XLT4 plates from BPW pre-enriched samples incubated in RV at 37 °C had moderate to heavy growth of competing organisms, whereas the paired 42 °C samples had much less growth of competing organisms. This qualitative difference in the growth of competing organisms was not evident for the TTB enriched cultures, with relatively light growth of competing organisms seen at both temperatures.

Delayed secondary enrichment

Combining the two experiments which compared delays of 0, 4 and 8 days before secondary enrichment, *Salmonella* bacteria were isolated from a total of 71 of 178 (40%) faecal samples. Of these, 50 were culture-positive for *Salmonella* bacteria after primary enrichment alone, 46 after secondary enrichment without delay (0 days), and 56 and 51 samples were culture-positive for *Salmonella* bacteria after 4 and 8 day delays, respectively. Combining results from the four experiments in which DSE after a 4-d delay was evaluated, single enrichment detected 78·4% of positive samples (positive by either SE or DSE), while DSE alone detected 197 (81·7%) of positive samples (Table 3). The data indicate that sensitivity for detecting positive samples would be improved by approximately 25% if DSE were performed on those samples that were culture-negative after primary enrichment.

Table 3 Numbers of 742 faecal cultures found to contain *Salmonella* bacteria after single enrichment (SE) or delayed secondary enrichment (DSE) with a 4 day delay

	SE +	SE –	Total
DSE +	145	52	197
DSE–	41	504	545
Total	189	556	742

DISCUSSION

Enrichment in selective media is a standard procedure for isolation of *Salmonella* bacteria from contaminated materials, and the relative performance of isolation methods is affected by the relative concentrations of *Salmonella* bacteria and competing organisms in the source material (Jameson 1962; D'aoust 1989; Busse 1995). In comparison with food samples, relatively few studies have evaluated methods for isolating *Salmonella* bacteria from swine faeces. The range of options that might be evaluated in methodological studies of *Salmonella* isolation is overwhelming, and the approach for this study was to focus effort on the methods used in the respective laboratories for recent epidemiological studies in swine. The similarity of the results obtained with the two methods and laboratories indicates that differences in laboratory procedures are unlikely to account for differences in the results reported in previous studies by our groups (Anon. 1997; Davies *et al.* 1997). This result is serendipitous because the methods have notable differences, including (i) weight of faecal sample (10 g *vs* approximately 1 g), (ii) pre-enrichment in BPW in Method 1 *vs* direct enrichment into TTB and Hajna broths, (iii) incubation of RV broth at 42 °C *vs* 37 °C, and (iv) plating on XLT4 agar alone *vs* plating on XLT4 and BGS agars. Based on kappa statistics, it is inferred that differences between the respective methods were comparable with inter-laboratory differences as sources of variation. The kappa statistic is widely used to assess agreement between observations in clinical medicine and other fields, but no previous example has been found of the use of this statistic for comparing culture methods for *Salmonella* bacteria. The conventional interpretation of kappa is that values less than 0·4 indicate poor agreement between tests, 0·4–0·7 indicates reasonable agreement, and values greater than 0·7 indicate excellent agreement (Fleiss 1981). It is notable among these studies that the proportion of positive samples (i.e. positive on any culture) detected by any one method ranged from 55 to 74%. This is similar to estimates of relative sensitivity observed in other studies (O'Carroll *et al.* 1999; Funk *et al.* 2000), and reflects the generally unreliable nature of bacteriological culture of individual faecal samples.

The effect of faecal sample weight on detection of *Salmonella* bacteria has been largely ignored. However, a marked increase in *Salmonella* detection has been demonstrated with increasing sample weights ranging from rectal swabs (estimated 0·5 g) to 25 g faeces (Funk *et al.* 2000). This effect was again evident using Method 1 in this study. Results with 10 g faecal samples in Method 1 were comparable with results from approximately 1 g samples in Method 2, and Method 2 was markedly superior to Method 1 when compared on samples of equal weight. The likely effects of varying sample weights need to be considered when interpreting studies that employ different sample weights for comparing enrichment methods (Skovgaard *et al.* 1985). Further studies of the effect of faecal sample weight using direct enrichment of swine faeces in TTB are indicated, and researchers restricted to the use of small samples (e.g. in studies of piglets) should not use the Method 1 protocol.

Pre-enrichment is conventionally recommended for materials, such as food and environmental samples, likely to have low numbers of *Salmonella* bacteria that may have been stressed or injured by factors such as thermal or osmotic shock, or freezing and thawing (D'aoust 1989). The case for pre-enrichment of faecal samples is less clear, and it has been suggested that pre-enrichment of heavily contaminated materials may be counterproductive (Aho 1992). However, pre-enrichment of swine faecal samples has become a common practice, despite an apparent lack of studies of its efficacy (Anon. 1993; Hoofar and Baggessen 1998). Pre-enrichment appears to have been adopted somewhat empirically with swine faecal samples, and studies comparing enrichment broths for isolating *Salmonella* bacteria from swine faeces have failed to address the potential impact of pre-enrichment on the results obtained. Use of BPW pre-enrichment with faecal samples may be linked to the popularity of RV enrichment broth, which requires small inoculum volumes, or high inoculum ratios (Harvey and Price 1980). Harvey and Price (1981) considered the need for high inoculum ratios to be an advantage when pre-enrichment is used but, citing the example of pig faeces, a potential liability if direct inoculation with large amounts of material is desirable.

A relatively small number of previous studies comparing enrichment broths with swine faecal (or caecal) samples has reported conflicting findings (Skovgaard *et al.* 1985; Vassiliadis *et al.* 1987; Bager and Petersen 1991; Cherrington and Huis in't Veld 1993). All these studies compared RV broth with Muller–Kaufmann tetrathionate broth (MKTB), and some also included selenite broth (SB). Interestingly, the two studies that found RV broth to

give inferior results used direct inoculation of faeces into MKTB and selenite broth, and BPW pre-enrichment for RV samples (Skovgaard *et al.* 1985; Cherrington and Huis in't Veld 1993). In contrast, the two studies reporting superior results with RV broth used pre-enrichment in BPW for all samples (Vassiliadis *et al.* 1987; Bager and Petersen 1991). Curiously, the more recent studies (Bager and Petersen 1991; Cherrington and Huis in't Veld 1993) did not cite the preceding studies identified here and consequently, there has been no previous effort to reconcile these contradictory findings. The present data, on samples of equal weight, indicate that double selective enrichment (TTB/RV or Hajna/RV) yielded significantly superior detection than did non-selective pre-enrichment in BPW followed by RV enrichment. This observation is consistent with some previously published work (Skovgaard *et al.* 1985; Cherrington and Huis in't Veld 1993) and it is suggested that the apparent contradictions in other previous studies are attributable to the use of BPW pre-enrichment prior to enrichment in tetrathionate broths (Bager and Petersen 1991; Cherrington and Huis in't Veld 1993). Recently, Kim *et al.* (1999) reported that recovery of *Salmonella* bacteria from swine faeces enriched in TTB tended to be reduced if pre-enrichment in BPW was used. Studies with a range of materials have commonly found RV to be superior to tetrathionate broths when compared subsequent to pre-enrichment (Harvey and Price 1981; Rhodes and Quesnel 1986). The nature of the source material, the prevalent serotypes in the material, and the use of direct enrichment or pre-enrichment, were recognized to be factors likely to influence the results of studies comparing enrichment media (Harvey and Price 1981).

While enrichment for a period of 24 h is common, reports indicate that detection can be improved by increasing the duration of enrichment (prolonged enrichment), or by using two enrichment steps in series, or by secondary enrichment (Jameson 1962; Vassiliadis *et al.* 1970; Trichopoulos *et al.* 1972; D'aoust 1989). In a study using faecal samples from naturally infected pigs, prolonged enrichment (48 h) was advantageous when using MKTB and SB broths, but did not significantly increase detection of *Salmonella* bacteria in RV broth, compared with 24 h enrichment (Bager and Petersen 1991). These results support the respective practices employed in our laboratories when enriching in RV (24 h) or TTB (48 h). The present data also support incubation at 42 °C, rather than 37 °C, of RV broth from samples pre-enriched in BPW. However, it should be pointed out that due to the use of a water-bath for the 42 °C incubation instead of an air incubator (37 °C), a more rapid attainment of enrichment temperature and a more stable temperature may have contributed to the advantage seen at 42 °C.

Delayed secondary enrichment has also been effective in increasing detection of *Salmonella* bacteria in samples from poultry (Rigby and Pettit 1980; Waltman *et al.* 1991,1993) and other sources, including swine faeces (Nietfield *et al.* 1998; O'Carroll *et al.* 1999). The observation that secondary enrichment without delay (0 days delay) yielded the lowest recovery of *Salmonella* bacteria is consistent with the view that the beneficial effects reported with DSE are attributable to the period of room temperature storage rather than to a second enrichment step. Most previous studies of DSE have used TTB (Rigby and Pettit 1980; Waltmann *et al.* 1991, 1993), and the present data using RV broth indicate a comparable increase in sensitivity of the order of 25%. The favourable performance of XLT4 agar compared with brilliant green agar is consistent with earlier findings (Davies *et al.* 1997), indicating that single plating on XLT4 agar alone detected over 90% of samples found to contain *Salmonella* bacteria using double plating on both XLT4 and brilliant green agars.

In reviewing the literature on detection of *Salmonella* bacteria, an inescapable conclusion is that increased diagnostic endeavour, be it through more intensive sampling or the use of multiple enrichment broths or plating media, will yield increased detection (Harvey and Price 1967; D'aoust 1989). Design of epidemiological studies generally entails some compromise due to logistic and financial constraints. Investigators need to weigh the marginal cost of increased diagnostic effort against the expected marginal gain in sensitivity of detection, in relation to the objectives of their studies. It would be imprudent to recommend standardized bacteriological procedures for studies that are likely to have different aims and constraints. However, haphazard adoption by different investigators of the almost countless methodological options does present a barrier to reconciliation of findings from different studies. The fortuitous result that the different methods used in our respective laboratories yielded similar results provides a potential benchmark for other swine researchers in the USA. It is suggested that adoption of methods for isolating *Salmonella* bacteria from swine faeces which differ from those evaluated in this study should be accompanied by some assessment of their relative sensitivity for detecting *Salmonella* bacteria, compared with one of the methods described here. Such a framework would provide flexibility for all researchers yet maintain some basis for comparison of results from different studies.

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